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METHODS AND COMPOSITIONS
FOR THE TREATMENT OF PANCREATITIS

This application is a continuation-in-part of
10 application serial no. 09/288,326, filed April 8, 1999.

Field of the Invention

The present invention includes methods and
15 compositions for the treatment of acute pancreatitis.
In a preferred embodiment the invention concerns the use
of agents to reduce or prevent the secretion of
pancreatic digestive enzymes within the pancreas. Such
agents are targeted to pancreatic cells, and serve to
20 prevent the exocytotic fusion of vesicles containing
these enzymes with the plasma membrane. The invention
is also concerned with methods of treating a mammal
suffering from pancreatitis through the administration
of such agents.

25

Background of the Invention

Pancreatitis is a serious medical condition
involving an inflammation of the pancreas. In acute or
30 chronic pancreatitis the inflammation manifests itself
in the release and activation of pancreatic enzymes
within the organ itself, leading to autodigestion. In
many cases of acute pancreatitis, the condition can lead
to death.

35 In normal mammals, the pancreas, a large gland
similar in structure to the salivary gland, is
responsible for the production and secretion of

5 digestive enzymes, which digest ingested food, and
bicarbonate for the neutralization of the acidic chyme
produced in the stomach. The pancreas contains acinar
cells, responsible for enzyme production, and ductal
cells, which secrete large amounts of sodium bicarbonate
10 solution. The combined secretion product is termed
"pancreatic juice"; this liquid flows through the
pancreatic duct past the sphincter of Oddi into the
duodenum. The secretion of pancreatic juice is
stimulated by the presence of chyme in the upper
15 portions of the small intestine, and the precise
composition of pancreatic juice appears to be influenced
by the types of compounds (carbohydrate, lipid, protein,
and/or nucleic acid) in the chyme.

The constituents of pancreatic juice includes
20 proteases (trypsin, chymotrypsin, carboxypolypeptidase),
nucleases (RNase and DNase), pancreatic amylase, and
lipases (pancreatic lipase, cholesterol esterase and
phospholipase). Many of these enzymes, including the
proteases, are initially synthesized by the acinar cells
25 in an inactive form as zymogens: thus trypsin is
synthesized as trypsinogen, chymotrypsin as
chymotrypsinogen, and carboxypolypeptidase as
procarboxypolypeptidase. These enzymes are activated
according to a cascade, wherein, in the first step,
30 trypsin is activated through proteolytic cleavage by the
enzyme enterokinase. Trypsinogen can also be
autoactivated by trypsin; thus one activation has begun,
the activation process can proceed rapidly. Trypsin, in
turn, activates both chymotrypsinogen and
35 procarboxypolypeptidase to form their active protease
counterparts.

5 The enzymes are normally activated only when they
enter the intestinal mucosa in order to prevent
autodigestion of the pancreas. In order to prevent
premature activation, the acinar cells also co-secrete a
trypsin inhibitor that normally prevents activation of
10 the proteolytic enzymes within the secretory cells and
in the ducts of the pancreas. Inhibition of trypsin
activity also prevents activation of the other
proteases.

15 Pancreatitis can occur when an excess amount of
trypsin saturates the supply of trypsin inhibitor.
This, in turn, can be caused by underproduction of
trypsin inhibitor, or the overabundance of trypsin
within the cells or ducts of the pancreas. In the
latter case, pancreatic trauma or blockage of a duct can
20 lead to localized overabundance of trypsin; under acute
conditions large amounts of pancreatic zymogen secretion
can pool in the damaged areas of the pancreas. If even
a small amount of free trypsin is available activation
of all the zymogenic proteases rapidly occurs, and can
25 lead to digestion of the pancreas (acute pancreatitis)
and in particularly severe cases to the patient's death.

 Pancreatic secretion is normally regulated by both
hormonal and nervous mechanisms. When the gastric phase
of stomach secretion occurs, parasympathetic nerve
30 impulses are relayed to the pancreas, which initially
results in acetylcholine release, followed by secretion
of enzymes into the pancreatic acini for temporary
storage.

 When acid chyme thereafter enters the small
35 intestine, the mucosal cells of the upper intestine
release a hormone called secretin. In humans, secretin

5 is a 27 amino acid (3400 Dalton) polypeptide initially
produced as the inactive form prosecretin, which is then
activated by proteolytic cleavage. Secretin is then
absorbed into the blood. Secretin causes the pancreas
to secrete large quantities of a fluid containing
10 bicarbonate ion. Secretin does not stimulate the acinar
cells, which produce the digestive enzymes. The
bicarbonate fluid serves to neutralize the chyme and to
provide a slightly alkaline optimal environment for the
enzymes.

15 Another peptide hormone, cholecystokinin (CCK) is
released by the mucosal cells in response to the
presence of food in the upper intestine. As described
in further detail below, human CCK is synthesized as a
protoprotein of 115 amino acids. Active CCK forms are
20 quickly taken into the blood through the digestive
tract, and normally stimulate the secretion of enzymes
by the acinar cells. However, stimulation of the CCK
receptor by the CCK analogs cerulein and CCK-octapeptide
(CCK-8) appears to lead to a worsening of morbidity and
25 mortality in mammals in whom pancreatitis is induced.
See Tani et al., *Pancreas* 5:284-290 (1990).

As indicated above, the digestive enzymes are
synthesized as zymogens; proto-enzyme synthesis occurs
in the rough endoplasmic reticulum of the acinar cells.
30 The zymogens are then packaged within vesicles having a
single lipid bilayer membrane. The zymogens are packed
within the vesicles so densely that they appear as
quasi-crystalline structures when observed under light
microscopy and the zymogen granules are electron-dense
35 when observed under the electron microscope. The
vesicles are localized within the cytoplasm of the

5 acinar cells. Secretion of zymogens by the acinar cells occurs through vesicle docking and subsequent fusion with the plasma membrane, resulting in the liberation of the contents into the extracellular milieu.

Nerve cells appear to secrete
10 neurotransmitters and other intercellular signaling factors through a mechanism of membrane fusion that is shared with other cell types, see e.g., Rizo & Sudhof, *Nature Struct. Biol.* 5:839-842 (October 1998), hereby incorporated by reference herein, including the
15 pancreatic acinar cells.

Although the Applicants do not wish to be bound by theory, it is believed that a vesicle first contacts the intracellular surface of the cellular membrane in a reaction called docking. Following the docking step the
20 membrane fuses with and becomes part of the plasma membrane through a series of steps that currently remain relatively uncharacterized, but which clearly involve certain vesicle and membrane-associated proteins, as has been illustrated using neural models.

25 In neurons, neurotransmitters are packaged within synaptic vesicles, formed within the cytoplasm, then transported to the inner plasma membrane where the vesicles dock and fuse with the plasma membrane. Recent studies of nerve cells employing clostridial neurotoxins
30 as probes of membrane fusion have revealed that fusion of synaptic vesicles with the cell membrane in nerve cells depends upon the presence of specific proteins that are associated with either the vesicle or the target membrane. See *id.* These proteins have been
35 termed SNAREs. As discussed in further detail below, a protein alternatively termed synaptobrevin or VAMP

5 (vesicle-associated membrane protein) is a vesicle-associated SNARE (v-SNARE). There are at least two isoforms of synaptobrevin; these two isoforms are differentially expressed in the mammalian central nervous system, and are selectively associated with
10 synaptic vesicles in neurons and secretory organelles in neuroendocrine cells. The target membrane-associated SNAREs (t-SNAREs) include syntaxin and SNAP-25. Following docking, the VAMP protein forms a core complex with syntaxin and SNAP-25; the formation of the core
15 complex appears to be an essential step to membrane fusion. See Rizo & Sudhof, *id.* and Neimmann et al., *Trends in Cell Biol.* 4:179-185 (May 1994), hereby incorporated by referenced herein.

20 Recently evidence has increasingly indicated that the SNARE system first identified in neural cells is a general model for membrane fusion in eukaryotic cells. A yeast exocytotic core complex similar to that of the synaptic vesicles of mammalian neural cells has been characterized, and found to contain three proteins:
25 Sso 1 (syntaxin 1 homolog), SncI (synaptobrevin homolog), and sec9 (SNAP-25 homolog). Rizo & Sudhof, *id.* These proteins share a high degree of amino acid sequence homology with their mammalian synaptosomal counterparts.

30 All mammalian non-neuronal cells appear to contain cellubrevin, a synaptobrevin analog - this protein is involved in the intracellular transport of vesicles, and is cleaved by TeTx, BoNT/E, BoNT/F, and BoNT/G. Homologs of syntaxin have been identified in
35 yeast (e.g., sso1p and sso2p) and mammalian non-neuronal cells (syn2p, syn3p, syn4p and syn5p). Finally, as

5 indicated above, a yeast SNAP-25 homolog, sec9 has been identified; this protein appears to essential for vesicle fusion with the plasma membrane.

Intoxication of neural cells by clostridial neurotoxins exploits specific characteristics of the
10 SNARE proteins. These neurotoxins, most commonly found expressed in *Clostridium botulinum* and *Clostridium tetanus*, are highly potent and specific poisons of neural cells. These Gram positive bacteria secrete two related but distinct toxins, each comprising two
15 disulfide-linked amino acid chains: a light chain (L) of about 50 KDa and a heavy chain (H) of about 100 KDa, which are wholly responsible for the symptoms of botulism and tetanus, respectively.

The tetanus and botulinum toxins are among the most
20 lethal substances known to man; both toxins function by inhibiting neurotransmitter release in affected neurons.

The tetanus neurotoxin (TeNT) acts mainly in the central nervous system, while botulinum neurotoxin (BoNT) acts at the neuromuscular junction; both toxins
25 inhibit acetylcholine release from the nerve terminal of the affected neuron into the synapse, resulting in paralysis or reduced target organ function.

The tetanus neurotoxin (TeNT) is known to exist in one immunologically distinct type; the botulinum
30 neurotoxins (BoNT) are known to occur in seven different immunologically distinct serotypes, termed BoNT/A through BoNT/G. While all of these latter types are produced by isolates of *C. botulinum*, two other species, *C. baratii* and *C. butyricum* also produce toxins similar
35 to /F and /E, respectively. See e.g., Coffield et al.,
The Site and Mechanism of Action of Botulinum

5 *Neurotoxin in Therapy with Botulinum Toxin* 3-13
 (Jankovic J. & Hallett M. eds. 1994), the disclosure of
 which is incorporated herein by reference.

 Regardless of type, the molecular mechanism of
 intoxication appears to be similar. In the first step
10 of the process, the toxin binds to the presynaptic
 membrane of the target neuron through a specific
 interaction between the heavy chain and a neuronal cell
 surface receptor; the receptor is thought to be
 different for each type of botulinum toxin and for TeNT.

15 The carboxy terminal (C-terminal) half of the heavy
 chain is required for targeting of the toxin to the cell
 surface. The cell surface receptors, while not yet
 conclusively identified, appear to be distinct for each
 neurotoxin serotype.

20 In the second step, the toxin crosses the plasma
 membrane of the poisoned cell. The toxin is first
 engulfed by the cell through receptor-mediated
 endocytosis, and an endosome containing the toxin is
 formed. The toxin (or light chain thereof) then escapes
25 the endosome into the cytoplasm of the cell. This last
 step is thought to be mediated by the amino terminal (N-
 terminal) half of the heavy chain, which triggers a
 conformational change of the toxin in response to a pH
 of about 5.5 or lower. Endosomes are known to possess a
30 proton pump that decreases intra-endosomal pH. The
 conformational shift exposes hydrophobic residues in the
 toxin, which permits the toxin to embed itself in the
 endosomal membrane. The toxin then translocates through
 the endosomal membrane into the cytosol.

35 Either during or after translocation the disulfide
 bond joining the heavy and light chain is reduced, and

5 the light chain is released into the cytoplasm. The
entire toxic activity of botulinum and tetanus toxins is
contained in the light chain of the holotoxin; the light
chain is a zinc (Zn++) endopeptidase which selectively
cleaves the SNARE proteins essential for recognition and
10 docking of neurotransmitter-containing vesicles with the
cytoplasmic surface of the plasma membrane, and fusion
of the vesicles with the plasma membrane. The light
chain of TxNT, BoNT/B, BoNT/D, BoNT/F, and BoNT/G cause
specific proteolysis of VAMP, an integral protein.
15 During proteolysis, most of the VAMP present at the
cytosolic surface of the synaptic vesicle is inactivated
as a result of any one of these cleavage events. Each
toxin cleaves a different specific peptide bond.

BoNT/A and /E selectively cleave the plasma
20 membrane-associated SNARE protein SNAP-25; this protein
is bound to and present on the cytoplasmic surface of
the plasma membrane. BoNT/C1 cleaves syntaxin, which
exists as an integral protein having most of its mass
exposed to the cytosol. Syntaxin interacts with the
25 calcium channels at presynaptic terminal active zones.
See Tonello et al., *Tetanus and Botulism Neurotoxins in
Intracellular Protein Catabolism* 251-260 (Suzuki K &
Bond J. eds. 1996), the disclosure of which is
incorporated by reference as part of this specification.
30 Bo/NTC1 also appears to cleave SNAP-25.

Both TeNT and BoNT are specifically taken up by
cells present at the neuromuscular junction. BoNT
remains within peripheral neurons and, as indicated
above, blocks release of the neurotransmitter
35 acetylcholine from these cells.

By contrast TeNT, through its receptor, enters

5 vesicles that move in a retrograde manner along the axon
to the soma, and is discharged into the intersynaptic
space between motor neurons and the inhibitory neurons
of the spinal cord. At this point, TeNT binds receptors
of the inhibitory neurons, is again internalized, and
10 the light chain enters the cytosol to block the release
of the inhibitory neurotransmitters 4-aminobutyric acid
(GABA) and glycine from these cells. Id.

International Patent Publication No. WO 96/33273
relates to derivatives of botulinum toxin designed to
15 prevent neurotransmitter release from sensory afferent
neurons to treat chronic pain. Such derivatives are
targeted to nociceptive neurons using a targeting moiety
that binds to a binding site of the surface of the
neuron.

20 International Patent Publication No. 98/07864
discusses the production of recombinant toxin fragments
that have domains that enable the polypeptide to
translocate into a target cell or which increase the
solubility of the polypeptide, or both.

25

Summary of the Invention

The present invention concerns methods and
30 compositions useful for the treatment of acute
pancreatitis. This condition is largely due to the
defective secretion of zymogen granules by acinar cells,
and by the premature co-mingling of the secreted
zymogens with lysosomal hydrolysates capable of
35 activating trypsin, thereby triggering the protease
activation cascade and resulting in the destruction of

5 pancreatic tissue.

In one embodiment of this aspect, the invention is a therapeutic agent comprising a chimeric protein containing an amino acid sequence-specific endopeptidase activity which will specifically cleave at least one
10 synaptic vesicle-associated protein selected from the group consisting of SNAP-25, syntaxin or VAMP, in combination with the translocation activity of the N-terminus of a clostridial neurotoxin heavy chain, wherein the chimeric protein further comprises a
15 recognition domain which will bind a human cholecystokinin (CCK) receptor. Upon binding of the recognition domain of the protein to the CCK receptor, the protein is specifically transported into cells containing CCK receptors (pancreatic acinar cells)
20 through receptor-mediated endocytosis. In a preferred embodiment, the CCK receptor is the CCK A receptor.

Once inside the acinar cell, the chimeric protein functions in a manner similar to that of a clostridial neurotoxin within its target neuron. The toxin moiety
25 is translocated from the endosome into the cytoplasm, where it acts to cleave a SNARE protein identical or homologous to SNAP-25, syntaxin or VAMP. The cleavage of this protein prevents formation of a core complex between the SNARE proteins and thus prevents or reduces
30 the extent of fusion of the vesicle with the target membrane. This, in turn, results in inhibition of zymogen release from the acinar cells and of zymogen activation by lysosomal hydrolases. The autodigestion of pancreatic tissue in acute pancreatitis is therefore
35 reduced or eliminated.

Another embodiment of the present invention

5 concerns a method of treating a patient suffering from acute pancreatitis by administering an effective amount of such a chimeric protein.

Another embodiment of the invention concerns a therapeutic composition that contains the translocation
10 activity of a clostridial neurotoxin heavy chain in combination with a recognition domain able to bind a specific cell type and a therapeutic element having an activity other than the endopeptidase activity of a clostridial neurotoxin light chain. A non-exclusive list
15 of certain such therapeutic elements includes: hormones and hormone-agonists and antagonists, nucleic acids capable of being used as replication, transcription, or translational templates (e.g., for expression of a protein drug having the desired
20 biological activity or for synthesis of a nucleic acid drug as an antisense agent), enzymes, toxins, and the like.

In a preferred embodiment, the specific cell type is a pancreatic cell, most preferably a pancreatic
25 acinar cell.

Another embodiment is drawn to methods for the treatment of acute pancreatitis comprising contacting an acinar cell with an effective amount of a composition comprising a chimeric protein containing an amino acid
30 sequence-specific endopeptidase activity which will specifically cleave at least one synaptic vesicle-associated protein selected from the group consisting of SNAP-25, syntaxin or VAMP, in combination with the translocation activity of the N-terminus of a
35 clostridial neurotoxin heavy chain, wherein the chimeric protein further comprises a recognition domain able to

5 bind to a cell surface protein characteristic of an
human pancreatic acinar cell. Preferably the cell
surface protein is a CCK receptor protein; most
preferably the protein is the human CCK A protein. CCK
receptors (CCK-A receptor and CCK-B receptor) are found
10 mainly in on the surface of pancreatic acinar cells,
although they are also found in some brain cells and, to
a lesser extent on the surface of gastrointestinal
cells.

Any suitable route of administration may be used in
15 this aspect of the invention. Applicants currently
prefer to administer the therapeutic agent in an
intravenous infusion solution; however methods such as
ingestion (particularly when associated with neurotoxin-
associated proteins (NAPs); see Sharma et al., *J. Nat.*
20 *Toxins* 7:239-253(1998), incorporated by reference
herein), direct delivery to the pancreas, injection and
the like may also be used. The agent is substantially
specifically targeted to pancreatic cells; when the
agent contains a CCK receptor-binding domain, the blood-
25 brain barrier prevents the agent from interacting with
brain cells.

In yet another embodiment the invention provides a
composition comprising a drug or other therapeutic agent
having an activity other than that of a clostridial
30 neurotoxin light chain for intracellular delivery, said
agent joined to the translocation domain of a
clostridial neurotoxin heavy chain and a binding element
able to recognize a cell surface receptor of a target
cell. In a preferred embodiment, the target cell is not
35 a neuron. Also, in this embodiment it is preferred that
the drug or other therapeutic agent has an enzymatic,

5 catalytic, or other self-perpetuating mode of activity,
so that the effective dose of drug is greater than the
number of drug molecules delivered within the target
cell. A non-exclusive list of certain such drugs would
include: hormones and hormone-agonists and antagonists,
10 nucleic acids capable being of being used as
replication, transcription, or translational templates
(e.g., for expression of a protein drug having the
desired biological activity or for synthesis of a
nucleic acid drug as an antisense agent), enzymes,
15 toxins (such as diphtheria toxin or ricin), and the
like.

In this embodiment the drug may be cleavably linked
to the remainder of the composition in such a way as to
allow for the release of the drug from the composition
20 within the target cell.

The presently claimed compositions may be provided
to the patient by intravenous administration, may be
administered during surgery, or may be provided
parenterally.

25 WO 95/32738, which shares ownership with the
present application, describes transport proteins for
the therapeutic treatment of neural cells. This
application is incorporated by reference herein as part
of this specification.

30

Detailed Description of the Preferred Embodiments

In a basic and presently preferred form, the
invention comprises a therapeutic polypeptide comprising
35 three features: a binding element, a translocation
element, and a therapeutic element.

5 The binding element is able to bind to a specific target cell provided that the target cell is not a motor neuron or a sensory afferent neuron. Preferably, the binding element comprises an amino acid chain; also an independently, it is preferably located at or near the C-terminus of a polypeptide chain. By "binding element" is meant a chemical moiety able to preferentially bind to a cell surface marker characteristic of the target cell under physiological conditions. The cell surface marker may comprise a polypeptide, a polysaccharide, a lipid, a glycoprotein, a lipoprotein, or may have structural characteristics of more than one of these. By "preferentially interact" is meant that the disassociation constant (K_d) of the binding element for the cell surface marker is at least one order of magnitude less than that of the binding element for any other cell surface marker. Preferably, the disassociation constant is at least 2 orders of magnitude less, even more preferably the disassociation constant is at least 3 orders of magnitude less than that of the binding element for any other cell surface marker to which the therapeutic polypeptide is exposed. Preferably, the organism to be treated is a human.

In one embodiment the cell surface receptor comprises the histamine receptor, and the binding element comprises an variable region of an antibody which will specifically bind the histamine receptor.

In an especially preferred embodiment, the cell surface marker is a cholecystokinin (CCK) receptor. Cholecystokinin is a bioactive peptide that functions as both a hormone and a neurotransmitter in a wide variety of physiological settings. Thus, CCK is involved in the

5 regulation of gall bladder contraction, satiety, gastric emptying, and gut motility; additionally it is involved in the regulation of pancreatic exocrine secretion.

There are two types of CCK receptors, CCK A and CCK B; the amino acid sequences of these receptors have been
10 determined from cloned cDNA. Despite the fact that both receptors are G protein-coupled receptors and share approximately 50% homology, there are distinct differences between their physiological activity. The CCK A receptor is expressed in smooth muscle cells of
15 the gall bladder, smooth muscle and neurons within the gastrointestinal tract, and has a much greater affinity ($>10^2$ times higher) for CCK than the related peptide hormone gastrin. The CCK B receptor, found in the stomach and throughout the CNS, has roughly equal
20 ability to bind CCK and gastrin.

The varied activities of CCK can be partly attributed to the fact that CCK is synthesized as procholecystokinin, a protoprotein of 115 amino acids, and is then post-translationally cleaved into a number
25 of active fragments all sharing the same C-terminus. The amino acid sequence of human procholecystokinin is shown below; amino acid residues not present in the biologically active cleavage products are in lower case.

All amino acid sequences herein are shown from N-
30 terminus to C-terminus, unless expressly indicated otherwise:

Human procholecystokinin, having the amino acid sequence SEQ ID NO:1:

35

5 mmsgvclcvlmavlaagaltqpvp padpagsglqraeeaprrqlr VSQRT
DGESRAHLGA LLARYIQQAR KAPSGRMSIV KNLQNLDP SH
RISDRDYMGW MDF grrsaeeyeyps

Biologically active cleavage products of the full
10 length CCK chain include:
CCK-58, having the amino acid sequence SEQ ID NO:2:

VSQRT DGESRAHLGA LLARYIQQAR KAPSGRMSIV KNLQNLDP SH
RISDRDYMGW MDF;

15 CCK-39, having the amino acid sequence SEQ ID NO:
3:

YIQQAR KAPSGRMSIV KNLQNLDP SH RISDRDYMGW MDF;

20 CCK-33, having the amino acid sequence SEQ ID NO:
4:

KAPSGRMSIV KNLQNLDP SH RISDRDYMGW MDF;

25 CCK-12, having the amino acid sequence SEQ ID NO:
5:

ISDRDYMGW MDF;

30 and CCK-8, having the amino acid sequence SEQ ID
NO: 6:
RDYMGW MDF.

35 In each case, the biologically active polypeptides
contain post-translational modifications; in the case of

5 CCK species binding the CCK-A receptor, amidation of the
C-terminal phenylalanine, and sulfatation of the
tyrosine residue located seven residue from the C-
terminus of the biologically active species are required
for high affinity binding to the receptor. In the
10 case of CCK-B, only the C-terminal amidation is
necessary; sulfation of the tyrosine appears to make
little difference in CCK-B binding. These modifications
appear to be necessary for full biological activity,
although both the unmodified C-terminal pentapeptide and
15 tetrapeptide of CCK retains some biological activity.
Kennedy et al., *J. Biol. Chem.* 272: 2920-2926 (1997),
hereby incorporated by reference herein.

In a preferred embodiment, the biologically active
therapeutic polypeptide of the present invention
20 comprises a CCK binding element containing the post-
translational modifications described above. This
polypeptide can be produced by synthetic chemistry or,
preferably, can be produced by a combination of
recombinant and synthetic means using the "expressed
25 protein ligation" (EPL) method. See Cotton & Muir,
Chemistry & Biology 6:R247 (1999), hereby incorporated
by reference herein. In this method the therapeutic
polypeptide is expressed without the C-terminal binding
element as a fusion protein with an "intein" polypeptide
30 sequence positioned at the C-terminus thereof. The
intein comprises a conserved cysteine, serine, or
threonine residue at its amino terminus; the carboxyl
terminus of the intein contains a functional binding
sequence such as chitin binding domain (CBD), poly His
35 (6 or more consecutive histidine residues), or another
amino acid sequence capable of affinity binding. The

5 coding sequence of this recombinantly expressed
polypeptide is constructed using standard recombinant
DNA methods.

10 Additionally, standard solid phase peptide
synthesis methods are employed to construct a synthetic
peptide comprising a C-terminal amidated phenylalanine
and the desired CCK amino acid sequence. Such methods
are described in e.g., Bodansky, M. and Bodansky, A. *The
Practice of Peptide Synthesis* (2d ed. Trost B.M., ed.
Springer Laboratory 1994), hereby incorporated by
15 reference herein. The synthetic peptide also contains
an sulfated tyrosine at the position 7 residues from the
carboxyl terminus. This can be done either by
incorporation of commercially available Fmoc-Tyr(OSO₃⁻)-
OH into the peptide chain at the 7th amino acid position
20 prior to cleavage of the synthetic peptide from the
solid support hereby incorporated by reference herein),
or by standard peptide synthesis using tyrosine at
position 7, followed by a sulfation reaction of the
peptide resulting in tyrosine sulfate at the 7 position.
25 See e.g., Koeller, K.M., *J. Am. Chem. Soc.* 122:742-743
(2000). The synthetic peptide is constructed with a
cysteine (or serine or threonine) residue at the amino
terminus.

30 It will be understood that one can use either
hydroxyl-containing amino acids or cysteine as the amino
terminal residue of the intein and the synthetic
peptide, and either thiopheol, phenol or another
nucleophile capable of creating a reactive ester or
thioester linkage in accordance with the expressed
35 protein ligation methods described herein. However,

5 thiol-containing amino acid residues and thipheonol or
another sulfur-containing nucleophile are preferred.

Thus, according to one embodiment of the expressed
protein ligation method, the fusion protein is
immobilized following expression by incubation under
10 selective binding conditions with a surface to which the
binding partner of the carboxyl terminal has been joined
(e.g., where the binding moiety is CBP, the surface may
be a resin to which chitin is conjugated). The
immobilized fusion protein is then permitted to react in
15 a transthioesterification reaction with a S- or O-
containing reagent (such as thiophenol or phenol) and
the synthetic modified peptide described above. In this
-step, the intein which is joined to the carboxyl
terminus of the therapeutic polypeptide is cleaved at
20 the thioester (or ester) linkage, thus liberating the
protein from the surface to which it was bound. The
intein may be transiently replaced with the thiophenol
group, and the resulting thioester is then itself
attacked by the cysteine (or serine or threonine)
25 residue of the synthetic peptide; this reaction is then
spontaneously followed by a shift of the carbonyl bond
from S (or O) to the N terminal nitrogen of the
synthetic peptide, to form a peptide bond. The
resultant therapeutic polypeptide thus comprises a
30 threapeutic domain, a translocation domain, and a
binding domain comprising a CCK sequence modified to
contain the naturally occuring post-translational
modifications.

As intended herein, the term "extein" refers to a
35 portion of a chimeric polypeptide that borders one or
more intein, and is subsequently ligated to either

5 another extein or a synthetic polypeptide in the EPL
reaction referred to herein.

As intended herein, the term "intein" refers to a
portion of a chimeric polypeptide containing an N-
terminal cysteine, serine, or threonine which is excised
10 from said polypeptide during the EPL reaction referred
to herein.

Of course, the Applicants contemplate that this
method of producing a CCK-containing therapeutic
polypeptide is exemplary only, and that variations and
15 modification of the above-described method will be well
within the ability and knowledge of those of ordinary
skill in the art in light of the present patent
application.

While it will be understood that the applicants do
20 not wish to be bound by theory, the following findings
may assist an understanding the nature of the
interaction between CCK and the CCK receptors, and thus
between the CCK receptor binding element of an
embodiment of the present invention and its CCK receptor
25 target.

In pancreatic acinar cells the CCK A receptor
undergoes internalization to intracellular sites within
minutes after agonist exposure. Pohl et al., *J. Biol.*
Chem. 272: 18179-18184 (1997), hereby incorporated by
30 reference herein. The CCK B receptor has also shown the
same ligand-dependant internalization response in
transfected NIH 3T3 cells. In the CCK B receptor, but
not the CCK A receptor, the endocytotic feature of the
receptor been shown to be profoundly decreased by the
35 deletion of the C terminal 44 amino acids of the

5 receptor chain, corresponding in both receptors to an
cytoplasmic portion of the receptor chain.

Recent studies of the interaction between the CCK A
receptor and CCK have shown that the primary receptor
sequence region containing amino acid residues 38
10 through 42 is involved in the binding of CCK. Residues
Trp₃₉ and Gln₄₀ appear to be essential for the binding of
a synthetic CCK C-terminal nonapeptide (in which the
methionine residues located at residue 3 and 6 from the
C-terminus are substituted by norleucine and threonine
15 respectively) to the receptor. Kennedy et al., *supra*.
These residues do not appear to be essential for the
binding of CCK analogs JMV 180 (corresponding the
synthetic C-terminal heptapeptide of CCK in which the
phenylalanylamide residue is substituted by a
20 phenylethyl ester and the threonine is substituted with
norleucine), and JMV 179 (in which the phenylalanylamide
residue and the L-tryptophan residues of the synthetic
CCK nonapeptide are substituted by a phenylethyl ester
and D-tryptophan, respectively and the threonine is
25 substituted with norleucine). *Id.*

These and similar studies have shed light on the
structure of the CCK A receptor active site. Based on
receptor binding experiments, a current structural model
indicates that CCK residues Trp₃₀ and Met₃₁ (located at
30 positions 4 and 3, respectively, from the C terminus of
mature CCK-8) reside in a hydrophobic pocket formed by
receptor residues Leu₃₄₈, Pro₃₅₂, Ile₃₅₃ and Ile₃₅₆. CCK
residue Asp₃₂ (located at amino acid position 2 measured
from the C terminus of CCK-8) seems to be involved in an
35 ionic interaction with receptor residue Lys₁₁₅. CCK Tyr-

5 sulfate₂₇ (the CCK-8 residue 7 amino acids from C terminus) appears involved in an ionic interaction with receptor residue Lys₁₀₅ and a stacking interaction with receptor residue Phe₁₉₈. Ji, et al., 272 *J. Biol. Chem.* 24393-24401 (1997).

10 Such structural models provide detailed guidance to the person of ordinary skill in the art as to the construction of a variety of binding elements able to retain the binding characteristics of biologically active CCK peptides for the CCK-A receptor, for example,
15 as, for example, by site directed mutagenesis of a clostridial neurotoxin heavy chain. Similarly, models deduced using similar methodologies have been proposed for the CCK B receptor, see e.g., Jagerschmidt, A. et al., *Mol. Pharmacol.* 48:783-789 (1995), and can be used
20 as a basis for the construction of binding elements that retain binding characteristics similar to the CCK B receptor.

It will be appreciated that the CCK-B receptor is known to exist on the surface of neurons associated with
25 the certal nervous system. In one alternative embodiment of the present invention the therapeutic polypeptide may be directed (for example, by intrathecal application) to these neurons rather than to the pancreas); in such a case, the binding element may
30 comprise a CCK containing the C terminal amidation only.

Such a binding element may be constructed using the expressed protein ligation (EPL) methods described above. Indeed, EPL methods may be used to introduce and desired or required modifications to the therapeutic
35 element, the translocation element, and/or the binding element of the claimed therapeutic polypeptide.

5 Additionally, the binding element may comprise a
variable region of an antibody which will bind the CCK-A
or CCK-B receptor.

10 Nucleic acids encoding polypeptides containing such
a binding element may be constructed using molecular
biology methods well known in the art; see e.g.,
Sambrook et al., *Molecular Cloning: A Laboratory Manual*
(Cold Spring Harbor Laboratory Press 2d ed. 1989), and
expressed within a suitable host cell. The disclosure of
this latter reference is incorporated by reference
15 herein in its entirety.

20 The translocation element comprises a portion of a
clostridial neurotoxin heavy chain having a
translocation activity. By "translocation" is meant the
ability to facilitate the transport of a polypeptide
through a vesicular membrane, thereby exposing some or
all of the polypeptide to the cytoplasm.

25 In the various botulinum neurotoxins translocation
is thought to involve an allosteric conformational
change of the heavy chain caused by a decrease in pH
within the endosome.

30 This conformational change appears to involve and
be mediated by the N terminal half of the heavy chain
and to result in the formation of pores in the vesicular
membrane; this change permits the movement of the
proteolytic light chain from within the endosomal
vesicle into the cytoplasm. See e.g., Lacy, et al.,
Nature Struct. Biol. 5:898-902 (October 1998).

35 The amino acid sequence of the translocation-
mediating portion of the botulinum neurotoxin heavy
chain is known to those of skill in the art;
additionally, those amino acid residues within this

5 portion that are known to be essential for conferring
the translocation activity are also known.

It would therefore be well within the ability of
one of ordinary skill in the art, for example, to employ
the naturally occurring N-terminal peptide half of the
10 heavy chain of any of the various *Clostridium tetanus* or
Clostridium botulinum neurotoxin subtypes as a
translocation element, or to design an analogous
translocation element by aligning the primary sequences
of the N-terminal halves of the various heavy chains and
15 selecting a consensus primary translocation sequence
based on conserved amino acid, polarity, steric and
hydrophobicity characteristics between the sequences.
The therapeutic element of the present invention may
comprise, without limitation: active or inactive (i.e.,
20 modified) hormone receptors (such as androgen, estrogen,
retinoid, perioxysome proliferator and ecdysone
receptors etc.), and hormone-agonists and antagonists,
nucleic acids capable of being used as
replication, transcription, or translational templates
25 (e.g., for expression of a protein drug having the
desired biological activity or for synthesis of a
nucleic acid drug as an antisense agent), enzymes,
toxins (including apoptosis-inducing agents), and the
like.

30 In a preferred embodiment, the therapeutic element
is a polypeptide comprising a clostridial neurotoxin
light chain or a portion thereof retaining the SNARE-
protein sequence-specific endopeptidase activity of a
clostridial neurotoxin light chain. The amino acid
35 sequences of the light chain of botulinum neurotoxin
(BoNT) subtypes A-G have been determined, as has the

INS
DI

145
DI
5 amino acid sequence of the light chain of the tetanus
neurotoxin (TeNT). Each chain contains the Zn⁺⁺-binding
motif **His-Glu-x-x-His** (N terminal direction at the left)
characteristic of Zn⁺⁺-dependent endopeptidases (HELIH
in TeNT, BoNT/A /B and /E; HELNH in BoNT/C; and HELTH in
10 BoNT/D).

Recent studies of the BoNT/A light chain have
revealed certain features important for the activity and
specificity of the toxin towards its target substrate,
SNAP-25. Thus, studies by Zhou et al. *Biochemistry*
15 34:15175-15181 (1995) have indicated that when the light
chain amino acid residue His₂₂₇ is substituted with
tyrosine, the resulting polypeptide is unable to cleave
SNAP-25; Kurazono et al., *J. Biol. Chem.* 14721-14729
(1992) performed studies in the presynaptic cholinergic
20 neurons of the buccal ganglia of *Aplysia californica*
using recombinant BoNT/A light chain that indicated that
the removal of 10 N-terminal or 32 C-terminal residues
did not abolish toxicity, but that removal of 10 N-
terminal or 57 C-terminal residues abolished toxicity in
25 this system. Most recently, the crystal structure of
the entire BoNT/A holotoxin has been solved; the active
site is indicated as involving the participation of
His₂₂₂, Glu₂₂₃, His₂₂₆, Glu₂₆₁ and Tyr₃₆₅. Lacy et al., *supra*.
(These residues correspond to His₂₂₃, Glu₂₂₄, His₂₂₇, Glu₂₆₂
30 and Tyr₃₆₆ of the BoNT/A L chain of Kurazono et al.,
supra.) Interestingly, an alignment of BoNT/A through E
and TeNT light chains reveals that every such chain
invariably has these residues in positions analogous to
BoNT/A. Kurazono et al., *supra*.

5 The catalytic domain of BoNT/A is very specific for the C-terminus of SNAP-25 and appears to require a minimum of 16 SNAP-25 amino acids for cleavage to occur.

 The catalytic site resembles a pocket; when the light chained is linked to the heavy chain via the disulfide
10 bond between Cys₄₂₉ and Cys₄₅₃, the translocation domain of the heavy chain appears to block access to the catalytic pocket until the light chain gains entry to the cytosol. When the disulfide bond is reduced, the two polypeptide chains dissociate, and the catalytic
15 pocket is then "opened" and the light chain is fully active.

 As described above, VAMP and syntaxin are cleaved by BoNT/B, D, F, G and TeNT, and BoNT/C₁, respectively, while SNAP-25 is cleaved by BoNT/A and E.

20 The substrate specificities of the various clostridial neurotoxin light chains other than BoNT/A are known. Therefore, the person of ordinary skill in the art could easily determine the toxin residues essential in these subtypes for cleavage and substrate
25 recognition (for example, by site-directed mutagenesis or deletion of various regions of the toxin molecule followed by testing of proteolytic activity and substrate specificity), and could therefore easily design variants of the native neurotoxin light chain
30 that retain the same or similar activity.

 Additionally, construction of the therapeutic agents set forth in this specification would be easily constructed by the person of skill in the art. It is well known that the clostridial neurotoxins have three
35 functional domains analogous to the three elements of the present invention. For example, and without

5 limitation, the BoNT/A neurotoxin light chain is present
in amino acid residues 1-448 of the BoNT/A prototoxin
(i.e., before nicking of the prototoxin to form the
disulfide-linked dichain holotoxin); this amino acid
sequence is provided below as SEQ ID NO: 7. Active site
10 residues are underlined:

BoNT/A light chain (SEQ ID NO:7)

MPFVVKQFNKDPVNGVDIAYIKIPNAGQMOPVKAFKIHNKIWV
15 IPERDTFTNP EEGDLNPPPEAKQVPVSYDSTYLSTDNEKDNYLKGVTKLFERIYSTD
LGRMLLTSIVRGIPFWGGSTIDTELKVIDTNCINVIQPDGSYRSEELNLVIIGPSADI
IQFECKSFGHEVLNLTRNGYGSTQYIRFSPDFTFGFEESLEVDTNPLLGA GKFATDPA
VTLAHEL I H A G H R L Y G I A I N P N R V F K V N T N A Y Y E M S G L E V S F E E L R T F G G H D A K F I D S
20 LQENEFRLYYYNKFKDIASTLNKAKSIVGTTASLQYMKNVFKEKYLLSEDTSGKFSVD
KLKFDKLYKMLTEIYTEDNFVKFFKVLNRKTYLNFDAVFKINIVPKVNYTIYDGFNL
RNTNLAANFNGQNT EINNMF TKLKNFTGLFEFYKLLCVRGIITSKTKSLDKGYNK;

The heavy chain N-terminal (H_N) translocation
domain is contained in amino acid residues 449-871 of
25 the BoNT/A amino acid sequence, shown below as SEQ ID
NO: 8; a gated ion channel-forming domain probably
essential for the translocation activity of this peptide
is underlined (see Oblatt-Montal et al., *Protein Sci.*
4:1490-1497(1995), hereby incorporated by reference
30 herein.

ALNDLCIKVNNWDLFFSPSEDNFTNDLNKGEEITSDTNIEAAEENISLDLIQYYLTFNF
DNEPENIS IENLSSDIIGQLELMPN IERFPNGKKYELDKYTMFHYLRAQEF EHGKSRI
ALTNSVNEALLNPSRVYTFSSDYVKKVNKATEAAMFLGWVEQLVYDFTDETSEVSTT
35 DKIADITIIIPYIGPALNIGNMLYKDDFVGALIFSGAVILLEFIPEIAIPVLGTFALV
SYIANKVLTVQTIDNALS RNEKWDEVYKYIVTNWLAKVNTQIDLIRKKMKEALENQA
EATKAIINYQYNQYTEEEKNNINFNIDDLSSKLNESINKAMININKFLNQCSVSYLMN
SMIPYGVKRLDFDASLKDALLKYIYDNRGTLIGQVDR LKDKVNNTLSTDIPFQLSKY
VDNQRLLS TFTEYIK;

40

5 The heavy chain C-terminal neural cell binding domain is contained in amino acid residues 872-1296 (SEQ ID NO: 9) of the BoNT/A prototoxin.

10 NIINTSILNLRYESNHLIDLSRYASKINIGSKVNFDPIDKNQI
QLFNLESSKIEVILKNAIVYNSMYENFSTSWIRIPKYFNSISLNNEYTIINCMENNS
GWKVSILNYGEIIWTLQDTQEIKQRVVFYKYSQMINISDYINRWIFVTITNNRLNNSKIY
INGRLIDQKPISNLGNIHASNNIMFKLDGCRDTHRYIWIKYFNLFDKELNEKEIKDLY
DNQSNISGILKDFWGDYLYQYDKPYMLNLYDPNKYVDVNNVGIRGYMYLKGPRGSVMTT
15 NIYLNSSLYRGTKFIIKKYASGNKDNIVRNDRVYINVVVKNEYRLATNASQAGVEK
ILSALEIPDVGNLISQVVMKSKNDQGITNKCKMNLQDNNNGNDIGFIGFHQFNNIKLV
ASNWYNRQIERSSRTLGCSEFIPVDDGWERPL

The amino acid sequence of the BoNT/A prototoxin is encoded by nucleotides 358 to 4245 of the neurotoxin
20 cDNA sequence, set forth herein below as SEQ ID NO: 10.

aagcttctaa atttaaatta ttaagtataa atccaaataa acaatatggt
caaaaacttg
25 atgaggtaat aatttctgta ttagataata tggaaaaata tatagatata
tctgaagata
atagattgca actaatagat aacaaaaata acgcaaagaa gatgataatt
agtaatgata
tatttatttc caattgttta accctatctt ataacggtaa atatatatgt
ttatctatga
30 aagatgaaaa ccataattgg atgatatgta ataattgatat gtcaaagtat
ttgtatttat
ggtcatttaa ataattaata atttaattaa ttttaaatat tataagaggt
gttaaatatg
35 ccatttgta ataaacaatt taattataaa gatcctgtaa atgggtgtga
tattgcttat
ataaaaattc caaatgcagg acaaatgcaa ccagtaaaag cttttaaaat
tcataataaa
atatgggtta ttccagaaag agatacatctt acaaatcctg aagaaggaga
tttaaattcca
40 ccaccagaag caaaacaagt tccagtttca tattatgatt caacatattt
aagtacagat
aatgaaaaag ataattattt aaaggagggt acaaaaattat ttgagagaat
ttattcaact
gatcttgga gaatgttggt aacatcaata gtaaggggaa taccattttg
45 ggggtggaagt
acaatagata cagaattaaa agttattgat actaattgta ttaattgtat
acaaccagat
ggtagttata gatcagaaga acttaattcta gtaataatag gaccctcagc
tgatattata

5	cagtttgaat tggttatggc tctactcaat gtcacttgaa gttgatacaa agtaacatta gcacatgaac tccaaatagg gtttttaaag aagctttgag gaacttagaa gaaaaacgaa tttcgtctat taaagctaaa tcaatagtag agagaaatat ctcctatctg tgataagtta tacaaaatgt taaagtactt aacagaaaaa agtacctaag gtaaattaca agcaaacttt aatgggtcaaa ttttactgga ttgtttgaat aactaaatca ttagataaag taattgggac ttgtttttta agaagaaatt acatctgata aatacaacaa tattatttaa aaatcctttca agtgacatta taatggaaaa aagtatgagt atttgaacat ggtaaactca tcctagtcgt gtttatacat ggaggcagct atgttttttag tagcgaagta agtactacgg acctgcttta aatataggta ttcaggagct gttattctgt ttttgcactt	gtaaaagctt acatttagatt atcctctttt ttatacatgc taaataactaa catttggggg attattataa gtactactgc aagatacatc taacagagat catatttgaa caatatatga atacagaaat cttttaattt taggccaatt tagataaata ggatttgctt ttttttcttc gctgggtaga ataaaaattgc atatgttata tagaatttat	tggacatgaa tagcccagat aggtgcaggc tggacataga tgcctattat acatgatgca taagtttaaa ttcattacag tggaaaattt ttacacagag ttttgataaa tggatttaat taataatatg tgataatgaa agaacttatg aacaatttct ggatataact taaagatgat accagagatt	gttttgaatc tttacatttg aaatttgcta ttatatggaa gaaatgagtg aagtttatag gataattttg gccgtattta ttaagaaata aattttacta agaggggataa gatttatgta actaatgatc gaaaatatta cctgaaaata cctaatatag gttaacgaag aagaaagtta tatgatttta ataattattc tttgtaggtg gcaataacctg	ttacgcgaaa gtttttgagga cagatccagc tagcaattaa ggttagaagt atagtttaca gtacacttaa atgttttttaa aattaaaatt ttaagttttt agataaatat caaatttagc aactaaaaaa taactttctaa tcaaagttaa taaataaagg gttttagattt tttcaataga aaagatttcc gtgctcaaga cattattaaa ataaagctac ccgatgaaac catatatagg ctttaatatt tattaggtac
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5 gtatcatata ttgcgaataa ggttctaacc gttcaaacia tagataatgc
 ttttaagtaaa
 agaaatgaaa aatgggatga ggtctataaa tatatagtaa caaattgggt
 agcaaagggt
 aatacacaga ttgatctaata aagaaaaaaa atgaaagaag ctttagaaaa
 10 tcaagcagaa
 gcaacaaagg ctataataaa ctatcagtat aatcaatata ctgaggaaga
 gaaaaataat attaatTTTA atattgatga ttttaagttcg aaacttaatg
 agtctataaa taaagctatg attaatataa ataaattttt gaatcaatgc
 tctgtttcat atttaatgaa ttctatgatc
 15 ccttatgggtg ttaaacgggt agaagatttt gatgctagtc ttaaagatgc
 attattaaag
 tatatatatg ataataagagg aactttaatt ggtcaagtag atagattaaa
 agataaagtt
 aataatacac ttagtacaga tatacctttt cagctttcca aatacgtaga
 20 taatcaaaga
 ttattatcta catttactga atatattaag aatattatta atacttctat
 attgaattta
 agatatgaaa gtaatcattt aatagactta tctaggtatg catcaaaaat
 aaatattgggt
 25 agtaaagtaa attttgatcc aatagataaa aatcaaattc aattatttaa
 ttttagaaagt
 agtaaaattg aggtaatttt aaaaaatgct attgtatata atagtatgta
 tgaaaatttt
 agtactagct tttggataag aattcctaag tattttaaca gtataagtct
 30 aaataatgaa
 tatacaataa taaattgtat ggaaaataat tcaggatgga aagtatcact
 taattatgggt
 gaaataatct ggactttaca ggatactcag gaaataaaaac aaagagtagt
 ttttaaatac
 35 agtcaaataa ttaatatatc agattatata aacagatgga tttttgtaac
 tatcactaat
 aatagattaa ataactctaa aatttatata aatggaagat taatagatca
 aaaaccaatt
 tcaaatttag gtaatatcca tgctagtaat aatataatgt ttaaattaga
 40 tggttgtaga
 gatacacata gatatatattg gataaaatat tttaatcttt ttgataagga
 attaaatgaa
 aaagaaatca aagatttata tgataatcaa tcaaattcag gtatttttaa
 agacttttgg
 45 ggtgattatt tacaatatga taaaccatac tatatgttaa atttatatga
 tccaaataaa
 tatgtcgatg taaataatgt aggtattaga gggttatatgt atcttaaagg
 gcctagaggt
 agcgtaataa ctacaaacat ttattttaaat tcaagtttgt atagggggac
 50 aaaatttatt
 ataaaaaaat atgcttctgg aaataaagat aatattgtta gaaataatga
 tcgtgtatat
 attaatgtag tagttaaaaa taaagaatat aggttagcta ctaatgcac
 acaggcaggc
 55 gtagaaaaaa tactaagtgc attagaaata cctgatgtag gaaatctaag
 tcaagtagta

5 gtaatgaagt caaaaaatga tcaaggaata acaaataaat gcaaaatgaa
 ttacaagat
 aataatggga atgatatagg ctttatagga tttcatcagt ttaataatat
 agctaaacta
 gtagcaagta attggtataa tagacaaata gaaagatcta gtaggacttt
 10 ggggtgctca
 tgggaattta ttctgtaga tgatggatgg ggagaaaggc cactgtaatt
 aatctcaaac
 tacatgagtc tgtcaagaat tttctgtaa catccataaa aattttaaaa
 ttaatatgtt
 15 taagaataac tagatatgag tattgtttga actgcccctg tcaagtagac
 aggtaaaaaa
 ataaaaatta agatactatg gtctgatttc gatattctat cggagtcaga
 ccttttaact
 tttcttgat cctttttgta ttgtaaaact ctatgtattc atcaattgca
 20 agttccaatt
 agtcaaaatt atgaaacttt ctaagataat acatttctga ttttataatt
 tcccaaaatc
 cttccatagg accattatca atacatctac caactcgaga catactttga
 gttgcgccta
 25 tctcattaag tttattcttg aaagatttac ttgtatattg aaaaccgcta
 tcaactgtgaa
 aaagtggact agcatcagga ttggaggtaa ctgctttatc aaaggtttca
 aagacaagga
 cgttgttatt tgattttcca agtacatagg aaataatgct attatcatgc
 30 aatcaagta
 tttcactcaa gtacgccttt gtttcgtctg ttaac

Of course, three distinct domains analogous to
 those described above for BoNT/A exist for all the BoNT
 35 subtypes as well as for TeNT neurotoxin; an alignment of
 the amino acid sequences of these holotoxins will reveal
 the sequence coordinates for these other neurotoxin
 species. Additionally, while sequence information is
 given above for BoNT/A, the amino acid sequences of all
 40 BoNT species and tetanus toxin TeNT are known and can
 easily be obtained from, for example, the NCBI Gen-Bank
 Web site: www.ncbi.nlm.nih.gov. The Clostrdial
 neurotoxin nucleotide and amino acid sequences disclosed
 at this site are expressly incorporated by reference
 45 herein.

5 Preferably, the translocation element and the
binding element of the compositions of the present
invention are separated by a spacer moiety that
facilitates the binding element's binding to the desired
cell surface receptor. Such a spacer may comprise, for
10 example, a portion of the BoNT Hc sequence (so long as
the portion does not retain the ability to bind to the
BoNT or TeNT binding site of motor neurons or sensory
afferent neurons), another sequence of amino acids, or a
hydrocarbon moiety. The spacer moiety may also comprise
15 a proline, serine, threonine and/or cysteine-rich amino
acid sequence similar or identical to a human
immunoglobulin hinge region. In a preferred embodiment,
the spacer region comprises the amino acid sequence of
an immunoglobulin $\gamma 1$ hinge region; such a sequence has
20 the sequence (from N terminus to C terminus):

EPKSCDKTHTCPPCP (SEQ ID NO:11)

It will be understood that none of the examples or
embodiments described herein are to be construed as
limiting the scope of the invention, which is defined
25 solely by the claims that conclude this specification.

Example 1:

An agent for the treatment of acute pancreatitis is
30 constructed as follows.

A culture of *Clostridium botulinum* is permitted to
grown to confluence. The cells are then lysed and total
RNA is extracted according to conventional methods and
in the presence of an RNase inhibitor. The RNA
35 preparation is then passed over a oligo(dT) cellulose
column, the polyadenylated messenger RNA is permitted to

5 bind, and the column is washed with 5-10 column volumes
of 20 mM Tris pH 7.6, 0.5 M NaCl, 1 mM EDTA
(ethylenediamine tetraacetic acid), 0.1% (w/v) SDS
(sodium dodecyl sulfate). Polyadenylated RNA is then
eluted with 2-3 column volumes of STE (10 mM Tris (pH
10 7.6), 1 mM EDTA, 0.05% (w/v) SDS). The pooled mRNA is
then precipitated in 2 volumes of ice cold ethanol,
pelleted in a centrifuge at 10,000 x g for 15 minutes,
then redissolved in a small volume of STE.

The BoNT/A mRNA is used as a template for DNA
15 synthesis using Moloney murine leukemia virus reverse
transcriptase (MMLV-RT), then the L chain and then H_N
chain of the neurotoxin is amplified from the cDNA by
the polymerase chain reaction (PCR) using appropriate
oligonucleotide primers whose sequences are designed
20 based on the BoNT/A neurotoxin cDNA sequence of SEQ ID
NO: 9. These procedures are performed using the
standard techniques of molecular biology as detailed in,
for example, Sambrook et al., already incorporated by
reference herein. The primer defining the beginning of
25 the coding region (5' side of the L chain fragment) is
given a StuI site. The PCR primer defining the 3' end of
the H_N-encoding domain has the following features (from
3' to 5'): a 5' region sufficiently complementary to the
3' end of the H_N-encoding domain to anneal thereto under
30 amplification conditions, a nucleotide sequence encoding
the human immunoglobulin hinge region γ_1 (SEQ ID NO:11),
a nucleotide sequence encoding the human CCK-8
octapeptide (SEQ ID NO:6), and a unique restriction
endonuclease cleavage site.

5 The PCR product (termed BoNT/AL^{HN-γCCK}) is purified
by agarose gel electrophoresis, and cloned into a
pBluescript II SK vector. The resulting plasmid is used
to transform competent *E. coli* cells, and a preparation
of the resulting plasmid is made. The BoNT/AL^{HN-γCCK}
10 fragment is excised from the pBluescript vector and
cloned into a mammalian expression vector immediately
downstream of a strong promoter. The resulting vector
is used to transfect a culture of the appropriate host
cell, which is then grown to confluence. Expression of
15 the BoNT/AL^{HN-γCCK} polypeptide is induced, and the cells
are lysed. The polypeptide is first purified by gel
exclusion chromatography, the fractions containing the
recombinant therapeutic agent are pooled, then the
BoNT/AL^{HN-γCCK} polypeptide is further purified using an
20 anti-Ig affinity column wherein the antibody is directed
to the γ₁ hinge region of a human immunoglobulin.

5 Example 2: Method of Treating a Patient Suffering from
 Acute Pancreatitis

 A therapeutically effective amount of the BoNT/A<sup>LHN-
10 Y-CKK</sup> agent constructed and purified as set forth in
Example 1 is formulated in an acceptable infusion
solution. Properties of pharmacologically acceptable
infusion solutions, including proper electrolyte
balance, are well known in the art. This solution is
provided intravenously to a patient suffering from acute
15 pancreatitis on a single day over a period of one to two
hours. Additionally, the patient is fed intravenously
on a diet low in complex carbohydrates, complex fats and
proteins.

 At the beginning of treatment, the patient's
20 pancreas shows signs of autodigestion, as measured by
blood amylase levels. After the treatment regimen,
autodigestion has ceased, and the patient's pancreas has
stabilized.

25 Example 3: Alternative Treatment Method

 In this example, a patient suffering from acute
pancreatitis is treated as in Example 2, with, the
therapeutic agent given continuously over a period of
30 two weeks. After the treatment regimen, autodigestion
has ceased, and the patient's pancreas has stabilized.

Patent

It will be understood that the present invention is not to be limited by the embodiments and examples described herein, and that the invention is defined solely by the claims that conclude this specification.